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# Distribution of (1,3)(1,4)-β-D-Glucans in Grains of Polish Oat Cultivars and Lines (*Avena sativa* L.) – Short Report

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The distribution of a fraction of soluble dietary fibre (1,3)(1,4)-β-D-glucans was depicted in selected Polish oat grains (*Avena sativa* L.). Localisation of β-glucans within the grains was visualised by the light microscopy with Calcofluor white as a fluorescence agent. The content of β-glucans varied in samples from 3.08% d.m. to 5.04% d.m. Analysis of distribution of (1,3)(1,4)-β-D-glucans showed that the localization of β-glucans varied between various cultivars and lines. It was demonstrated that the total content of (1,3)(1,4)-β-D-glucans in oat kernels had an effect on their distribution. All the lines and cultivars tested displayed the greatest accumulation of (1,3)(1,4)-β-D-glucans in the cells of the subaleurone layer. With increase in the levels of β-glucans in high-glucan oat cultivars and lines, a tendency was observed towards their greater accumulation in the central parts of the kernel. It makes oat grain particularly suitable for the production of wholemeal foods. It is important not only to focus on increasing the content of β-glucans, but also to investigate molecules distribution in the seed. It was also demonstrated that Dukat cultivar was characterised by an especially valuable triple aleurone layer, which makes this cultivar predestined for further breeding studies as an extremely valuable carrier of genetic information.

## INTRODUCTION

The functional traits of oat products are largely affected by the physicochemical properties of dietary fibre. Oat dietary fibre, and especially its soluble fractions, lower the level of total cholesterol, and that of the LDL fraction with particular intensity [Queenan *et al*., 2007; Andersson *et al*., 2010]. Oat products can also be highly important elements of the prophylaxis and alleviation of disorders of carbohydrate metabolism [Weickert *et al*., 2006; Chang *et al*., 2013]. Note is also taken of the special role of prebiotic fractions of dietary fibre in the prophylaxis of neoplastic diseases of the large intestine [Sayar *et al*., 2007] and affecting the intestinal immune response [Volman *et al*., 2010; 2011]. It is emphasised that the physicochemical effects of soluble dietary fibre are more comprehensive and effective when it appears in its native structures, not damaged by processing [Delaney *et al*., 2003]. The health-promoting effects of dietary fibre are amplified additionally by a group of biologically-active food components related with fibre structures (so-called *copassangers*) [Liu *et al*., 2004].

Numerous studies show that (1,3)(1,4)-β-D-glucans are particularly active components of soluble dietary fibre, responsible for the health-promoting properties of oat products [Lazaridou *et al*., 2011; Zhao *et al*., 2014]. Many researchers also indicate an extensive and multi-factor variation in the content of (1,3)(1,4)-β-D-glucans in oat grain: in low-glucan cultivars that content is *ca*. 2.0% of d.m., while in high-glucan cultivars it may even exceed 8% of d.m. [Andersson & Börjesdotter, 2011; Redaelli *et al*., 2013; Sikora *et al*., 2013]. It is pointed out that the content of (1,3)(1,4)-β-D-glucans is determined primarily by the cultivar factor, although it has also been confirm to depend on environmental conditions [Andersson & Börjesdotter, 2011, Sikora *et al*., 2013]. (1,3)(1,4)-β-D-glucans are not uniformly distributed within the kernel. It was shown that localization of β-glucan varied between the different lines: their largest amounts appear in the outer layers of the kernel, mainly in the cell walls of the subaleurone and aleurone layers [Sikora *et al*., 2013]. In smaller amounts they are also present in kernel endosperm [Gajdošová *et al*., 2007]. Fulcher & Miller [1993] demonstrated that with an increase in the content of (1,3)(1,4)-β-D-glucans in oat grain an increase is also observed in the content of (1,3)(1,4)-β-D-glucans in the inner parts of the kernel. Therefore, the accumulation of those components in the subaleurone layer mainly, seems to be a property of cultivars with low levels of (1,3)(1,4)-β-D-glucans.

It is emphasised that the functional properties of oat food products, both hypocholesterolemic and hypoglycae-

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TABLE 1. Chemical composition of oat cultivars and lines (% d.m. ± SD).

| Material | (1,3)(1,4)-β-D-glucans | IDF[A] | SDF[B] | TDF[C] |
|---|---|---|---|---|
| Krezus | 4.61[b] ± 0.05 | 12.33[c] ± 0.01 | 6.8[a] ± 0.16 | 19.13[b] ± 0.17 |
| Bajka | 4.57[b] ± 0.18 | 14.1[b] ± 0.38 | 5.52[b] ± 0.11 | 19.62[b] ± 0.49 |
| Dukat | 4.74[a,b] ± 0.04 | 14.04[b] ± 0.18 | 5.5[b] ± 0.02 | 19.54[b] ± 0.18 |
| STH 7205 | 5.04[a] ± 0.13 | 14.75[a] ± 0.17 | 6.59[a] ± 0.28 | 21.34[a] ± 0.45 |
| STH 6905 | 3.08[c] ± 0.14 | 11.19[d] ± 0.17 | 4.6[c] ± 0.07 | 15.79[c] ± 0.24 |

[A]insoluble dietary fiber. [B]soluble dietary fiber. [C]total dietary fiber. All analyses were performed in 3 replications. Statistical analysis was performed using the SAS 9.1.3 statistical software. One-way analysis of variance was made (Tuckey test). Means followed by different superscript within a column are significantly different ($p<0.05$).

mic, are also dependent on the physicochemical properties of (1,3)(1,4)-β-D-glucans [Åman *et al*., 2004]. Significant in this respect are their viscosity and molecular mass [Skendi *et al*., 2003; Colleoni-Sirghie *et al*., 2004]. Processing leads to a reduction of the molecular mass of (1,3)(1,4)-β-D-glucans and to deterioration of their capability to generate the viscosity of the gastric digesta [Kerckhoffs *et al*., 2003]. Serious alterations of the structure of (1,3)(1,4)-β-D-glucans may also take place during the storage of oat products [Gajdošová *et al*., 2007]. Beta-glucanase, naturally occurring in grain and activating as a result of kernel structure destruction during milling, affects the structure of (1,3)(1,4)-β-D-glucans, induces their hydrolysis, reduces their molecular mass and lowers their viscosity. Therefore, each technological process that causes a change in their molecular mass and deterioration of their functional properties should be limited to the necessary minimum [Kerckhoffs *et al*., 2003; Frank *et al.,* 2004].

Regarding the functional properties of (1,3)(1,4)-β-D--glucans it is important to investigate how these molecules are distributed in the seed. Correct design of high quality functional food requires not only raw materials with suitable chemical composition; but also proper selection of technological processes, adequate to the properties of the raw materials. Proper utilisation of oat material for the production of functional food shall take into account (1,3)(1,4)-β-D--glucans distribution in the kernel. Precise determination of their distribution in the particular cultivars is the prerequisite for correct selection of cultivars for the particular types of use and for proper design of technological processes of oat grain as well as for the preservation of the functional properties of the end product. The role of different oat products in modelling the properties of wheat flour, wheat-oat dough, baked products and quality of sponge-fatty cakes was investigated in many research. Bran additives are more beneficial than the oat flour added – the properties of wheat-oat doughs and breads were more favourably affected by oat flakes and bran than by oat flour [Czubaszek & Karolini-Skaradzińska, 2005]. The increase of the water--soluble dietary fiber fraction was observed when the oat flour was added to sponge cakes formula [Sobczyk, 2008]. The technology of oat bran production makes this product typically consisting of an enriched fraction of the aleurone layers. Cell walls from the starchy endosperm in practice are less important in bran production. All these parameters are of importance in further exploration of the physiological effects of different oat products based upon β-glucans content and distribution in the kernel.

Although the general localisation of β-glucans in oat and other cereals is well known, the knowledge of differences in its content and cellular localization in kernels of Polish oat cultivars can be useful to improve healthy properties of oat cultivars *via* breeding and thereby also to improve the quality of oat products. This study was aimed at determining the distribution of (1,3)(1,4)-β-D-glucans in kernels of oats of selected high-glucan Polish hulled cultivars and one low-glucan line of hulled oat.

## MATERIAL AND METHODS

### Plant material

The research material comprised 3 cultivars and 2 lines of oat (*Avena sativa* L.), originating from the experimental Plant Breeding Station in Strzelce (Poland). The crop was harvested in 2006.

### Methods

The contents of the total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) were determined with the enzymatic method [AACC, Method 32–07.01], using Megazyme enzymes and methodological procedures. Samples were cooked with heat stable α-amylase to induce gelatinisation, hydrolysis and depolymerisation of starch; incubated with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose); and treated with four volumes of ethanol to precipitate soluble fibre and remove depolymerised protein and glucose (from starch). The residue was filtered, washed with ethanol and acetone, dried and weighed. The content of (1,3)(1,4)--β-D-glucans was determined with the McCleary method [AACC Method 32–23], using Megazyme enzymes and methodological procedures. Samples were suspended and hydrated in a buffer solution of pH 6.5 and then incubated with purified lichenase enzyme and filtered. An aliquot of the filtrate was then hydrolysed to completion with purified β-glucosidase. The D-glucose produced was assayed using a glucose oxidase/peroxidase reagent. Calculations of (1,3)(1,4)-β-D-glucans content were made based on results of absorbance (510 nm).

The distribution of (1,3)(1,4)-β-D-glucans was visualised by light microscopy with Calcofluor white as fluorescent agent. Microscope specimens were prepared from cross-sec-

tions of oat kernels. They were fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH=7) at 4°C for 2 days. The specimens were rinsed with distilled water, dehydrated in ethanol series and sealed. The microscope specimens were cross-section sliced using a microtome and then the resultant slices were placed on microscope slides. After drying, the specimens were dyed with 0.01% Calcofluor white 2R and left to dry. After enclosing in the *DPX* medium, the dyed specimens were analysed under an OLYMPUS BX-60 microscope, at objective magnification of x20 and using an *NU* FC filter for the fluorescence technique.

## RESULTS AND DISCUSSION

The oat cultivars selected for the study had a high level of (1,3)(1,4)-β-D-glucans and dietary fibre (including soluble dietary fibre) (Table 1). The content of β-glucans varied from 4.57% d.m. to 5.04% d.m. and that of soluble dietary fiber from 5.5% d.m. to 6.8% d.m. For comparison, also included in the study was the STH 6905 line, with the lowest content of (1,3)(1,4)-β-D-glucans (3.08% d.m.) and the lowest level of soluble dietary fibre (4.6% d.m.).

The results of this study show that in general (1,3)(1,4)-β-D-glucans occur in the endosperm and in the subaleurone layer (Photo 1). The concentration of mixed linked β-glucans is not high in oat aleurone layer. The subaleurone layer is built of irregular cells that are notably larger than the aleurone cells, but smaller than the cells of the starch parenchyma. They show the most intense labeling compared to the aleurone cell walls, which indicates a high level of (1,3)(1,4)-β-D-glucans in those cellular structures. This regularity is observed in all of the oat cultivars under study (Photo 1). Differences in the intensity of labeling indicate the degree of their filling with (1,3)(1,4)-β-D-glucans, selectively reacting with the fluorescent agent. The cell walls of the subaleurone layer, forming the so-called outer parenchyma are an especially valuable element of the kernel. These layers can be separated from the starchy endosperm flour by conventional milling and sieving. This important structural feature allows production of bran fractions with as high as possible β-glucans content. The oat bran milling process involves cleaned and dehulled oat groats, which are sent through roll stands, where the bran is separated from the flour. This process results in two products – oat flour without bran and oat bran, where β-glucans have a great effect on its water-binding properties [Wood, 1993].

Note should be taken that the cultivars studied were grown under identical soil-climate and cultivation conditions. Line STH 6905 (Photo 1e), with the lowest content of (1,3)(1,4)-β-D-glucans (Table 1), is characterised by a notably weaker labeling compared to the remaining cultivars (Photo 1a,b,c,d). In line STH 6905, the largest amount of (1,3)(1,4)-β-D-glucans is located in the walls of subaleurone cells immediately adjacent to the aleurone layer. The relatively low β-glucan content in line STH 6905 makes it suitable only for limited number of functional food products, *e.g.* oat bran, which in fact is highly enriched with the thickened outermost cells of the endosperm (subaleurone cells). Although cell walls from the starchy endosperm are also present in the bran, this fraction is less important in bran production. In samples with higher levels of (1,3)(1,4)-β-D-glucans, Krezus, Bajka and line STH 7205 (Table 1), one can observe a more extensive area of intensive fluorescence (Photo 1a, c, d); intense labeling is localised at both the walls of cells immediately adjacent to the aleurone layer and those of cells of the subaleurone layer, closer to the proper parenchyma.

Careful analysis of Photo 1 allows also observing one more regularity. The cell walls of the subaleurone layer display a differentiation in their thickness. The greatest thickness, and thus the highest concentration of (1,3)(1,4)-β-D glucans, is observed at the point of contact of a cell with the aleurone layer. As the cells are located further away, towards the proper parenchyma, the intensity of labeling decreases.

According to Miller *et al*. [1995], (1,3)(1,4)-β-D-glucans and arabinoxylans are the dominant components of the cell walls of oat endosperm. With increasing distance from the subaleurone layer the cells show markedly reduced wall thickness. This regularity applies to all of the lines and cultivars studied (Photo 2). The weakest labeling was observed in the parenchyma cell walls of line STH 6905 (Photo 2e) - the line with the lowest content of (1,3)(1,4)-β-D-glucans. Thicker walls were noted in specimens of cultivars Dukat (Photo 2b), Krezus (Photo 2c), Bajka (Photo 2d) and line STH 7205 (Photo 2a).

Fulcher & Miller [1993] observed that the distribution of (1,3)(1,4)-β-D-glucans in oat kernels changes with increase in their total content. It was demonstrated that the cell walls of the proper parenchyma of the high-glucan cultivar Marion were thicker compared to the cells of the low-glucan cultivar OA516–2 [Miller & Fulcher, 1994]. Those observations indicate with special emphasis the sense of producing wholemeal oat products from oat variety with a high content of β–glucans. Different raw material is used in the production of oat flakes or rolled oats: groats or steel cut oats. Steel cut oats in different sizes are used to produce quick rolled oats and instant oatmeal. Whole groats produce old-fashioned types like regular, medium, and thick rolled oats. High-glucan oat variety should be used exclusively to produce whole oat flour from whole groats. Groats are fed to hammer mills, where they are converted into fine oat flour. The coarse flour, left after sifting, is again fed to the hammer mill, and this process continues. It gives products which are similar to oat grain in terms of the nutritional value.

The endosperm cell wall is an irregular border, wrapping around starch granules and protein bodies in the cell. Analysis of the fluorescence of parenchyma cells (Photo 2) revealed that (1,3)(1,4)-β-D-glucans were not uniformly distributed within the cell walls. Distinct, irregularly distributed swellings are observed on the boundary of the parenchyma cells. Cultivars with a high content of (1,3)(1,4)-β-D-glucans (Photo 2 a,b,c,d) display a greater concentration of irregular thicker fragments of cell walls compared to low-glucans line STH 6905 (Photo 2e). The thickness of the cell wall fragments in question is several-fold greater than that of the regular cell walls.

It is commonly believed that the aleurone layer in oat kernels is composed of a single layer of cells with shapes close

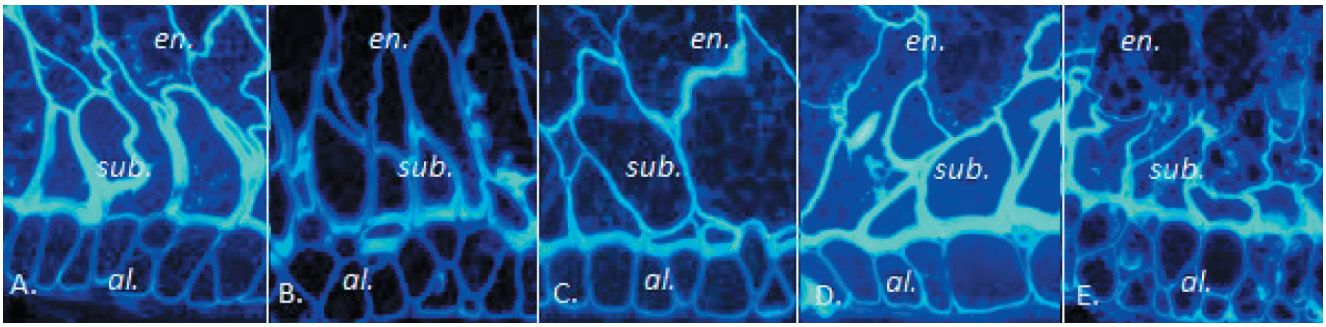


PHOTO 1. Distribution of (1,3)(1,4)-β-D-glucans in oat cultivars and lines (x20): A. STH 7205, B. Dukat, C. Krezus, D. Bajka, E. STH 6905 (*en* – endosperm, *sub* – subaleurone layer, *al* – aleurone layer).

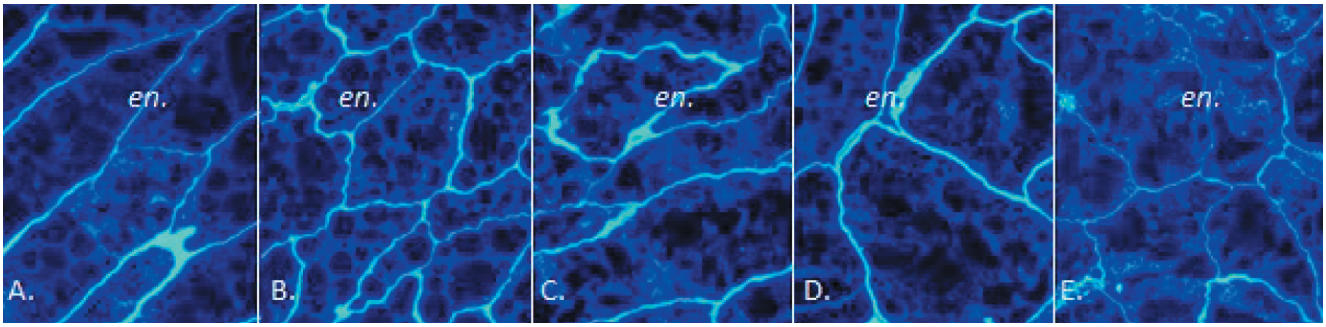


PHOTO 2. Endosperm (*en*) cells in oat grain cultivars and lines (20x): A. STH 7205, B. Dukat, C. Krezus, D. Bajka, E. STH 6905.

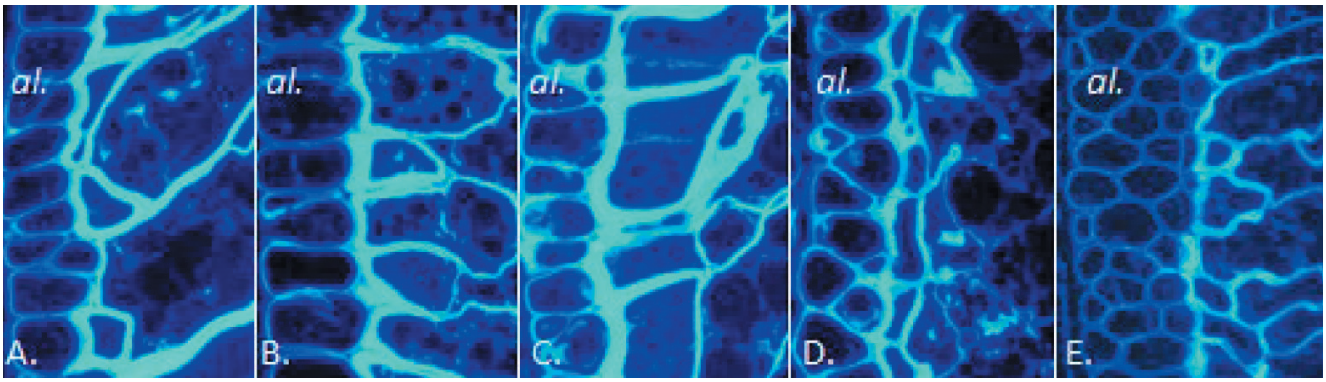


PHOTO 3. Aleurone layer (*al*.) in oat grain cultivars and lines: A. STH 7205, B. Krezus, C. Bajka, D. STH 6905, E. Dukat.

to the cuboid [Gąsiorowski & Cierniewska, 1995]. Each aleurone cell contains numerous individual protein bodies and each aleurone grain is surrounded by lipid droplets. The aleurone layer is important nutritionally because it is also a rich source of minerals, vitamins and other health-promoting compounds. Studies by Bechtel & Pomeranz [1981] prove that at certain points aleurone layer can increase its thickness to a double layer. Our study demonstrated that the oat cultivars and lines studied were characterised by varied structures of the aleurone layer (Photo 3). The aleurone layer in cultivars Krezus (Photo 3b), Bajka (Photo 3c) and lines STH 7205 (Photo 3a) and STH 6905 (Photo 3d) is formed by a distinct single chain of cells with the typical cuboid structure. A very interesting structure of the aleurone layer is observed in the case of cultivar Dukat (Photo 3e). The aleurone cells have irregular shapes and varied sizes. At points of deformation, the free space is filled with additional cells with notably smaller sizes. This creates an impression of the formation of a multi-layered aleurone layer. Aleurone cells form an irregular three-layered composition. One can, therefore, assume that such a cultivar with a multi-layered structure of the aleurone layer will be characterised by a potentially rich chemical composition, and especially by a high content of proteins. Our observation is supported by the analytical studies performed [Sykut-Domańska *et al*., 2013]. Cultivar Dukat is characterised by a very high protein content and can be an especially valuable raw material for the production of cereal functional foods. This cultivar should also be used as an especially valuable carrier of genes in further breeding programs.

The food processing industry should be supplied with oat cultivars with as high content of (1,3)(1,4)-β-D-glucans as possible. This study demonstrated that the primary technological discriminant determining the applicability of a given

cultivar for the production of functional food should not only be the content of functional components but also their distribution within the cross-section of kernels. Although levels of β-glucans are essential, it is also important to consider the localisation of the synthesised β-glucan in the seed. Analysis of distribution of (1,3)(1,4)-β-D-glucans in the cross-section of kernels should be an important indicator for the modelling cereal grain processing for food purposes. Such a distribution of the prebiotic fractions of dietary fibre makes high-glucan oat grain particularly suitable for the production of wholemeal foods.

## CONCLUSION

Significant differences were demonstrated in the distribution of (1,3)(1,4)-β-D-glucans in high-glucan and low-glucan oat cultivars.

The highest concentration of (1,3)(1,4)-β-D-glucans was found in the subaleurone layer; this regularity was noted in all oat cultivars and lines under study.

With increase in the content of (1,3)(1,4)-β-D-glucans their increased concentration is observed in the central areas of the parenchyma.

The cells of the proper parenchyma of high-glucan cultivars have thicker cell walls, filled with (1,3)(1,4)-β-D-glucans to a greater extent, compared to the cells of the low-glucan oat line.

Due to the distribution of (1,3)(1,4)-β-D-glucans, high-glucan oat cultivars are particularly useful for the production of wholemeal food products.

The characteristic triple-layered structure of the aleurone layer of cultivar Dukat makes it suitable for further breeding studies as an extremely valuable carrier of genetic information.

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